KILLFISH DEVELOPMENT IN ZERO-G ON COSMOS 782:

Fundulus Experiment K-104

by

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INTRODUCTION

The Kosmos K-104 Fundulus embryogenesis experiment was the third in a series of experiments to assess the possible effects of the space environment upon developing organisms. The experiment evolved from the Apollo Soyuz Test Project (ASTP) experiment MA-161. The major refinement lay in the incorporation of a 1-gravity onboard control treatment through the use of an onboard centrifuge. Other notable features were the employment of a low temperature transport system for retardation of development during shipment to the Soviet launch site and an adjunct Soviet experiment with clinostatted embryos.

EXPERIMENT BACKGROUND

The experimental organism was Fundulus heteroclitus (Walbaum) a small shallow-water minnow common to the Atlantic coast of North America. The Beaufort, North Carolina, strain employed in these experiments produces an egg having a transparent chorion thus allowing observation of development from first cleavage through hatching. The chorion is quite tough and the egg may be manipulated extensively without undue stress on the developing embryo. Because the biology and developmental relationships of the Fundulus are relatively well known (Armstrong & Child, 1965; Oppenheimer, 1937), and because of its physical attributes which allowed aseptic culture, the organism was used as a test organism at the NASA Lyndon B. Johnson Space Center (JSC) during the lunar quarantine. Subsequently the methodology for continous laboratory production of fertile eggs was developed in this laboratory and the accumulation of a data base for flight experimentation was initiated.

The initial Fundulus experiment was flown on the Skylab 3 mission. The experimental design was quite preliminary in form. The flight package consisted of a plastic bag containing 2 juvenile fish in a compartment with 250 milliliters of synthetic seawater (21 parts per thousand dissolved solids) and 50 fertilized eggs (5 days past fertilization) in a separate compartment with 50 milliliters of seawater. Initially, the juveniles exhibited obvious disorientation reactions (swimming rapidly in loops and circles), but over a period of several days in orbit, they gradually adapted to the weightless environment and to dependence on visual cues for orientation. In this state of adaptation, the locker door surface to which the plastic aquarium was attached served as "down". Adaptation was not complete, however, and occasional disoriented swimming activity occurred. Nearly all the 50 eggs hatched in space, and because of a delay in hatching of the flight fish, several returned alive to Earth. However, a series of unfortunate events resulted in the death of these animals shortly after return. The space-hatched Fundulus fry exhibited no observable tendency toward disoriented swimming acitvity, and their apparent dependence on visual

orientation cues both onboard the Skylab spacecraft and on the recovery ship suggested the possible absence of vestibular input. Preservation of the returned hatchlings was insufficient to prevent deterioration and did not permit definite conclusions regarding the condition of the vestibular system.

The second experiment in the series was the MA-161 experiment flown on the 9-day Apollo Soyuz Test Project mission to confirm and extend the observations of the Skylab experiment (Scheld et al., 1975, 1976). The experiment package consisted of two parts: a series of staged embryos in five individual compartments of a polyethylene bag and a series of preconditioned juvenile fish in a similar bag. Embryos at 32, 66, 128, 216, and 336 hours after fertilization at the time of launch were chosen to represent key stages of development; development occurred at a constant temperature of 295°K (22°C). Juvenile fish were reared from hatching for 21 days in specific visual environments. Experiment packages were mounted on the docking module wall and photographed periodically during the mission to record the swimming activity of the fish and the condition of the eggs. At splashdown and at selected times thereafter, juveniles and hatchlings were tested to assess normalcy of vestibular function and samples were fixed for microscopic examination to assess normalcy of anatomical development.

Juvenile fish in a null-gravity environment exhibited looping swimming activity similar to that observed during the Skylab 3 mission. Hatchlings from the 336-hour egg stage also were observed to loop. At splashdown, both juveniles and hatchlings exhibited a typical diving response suggesting relatively normal vestibular function. The juveniles exhibited swimming patterns suggestive of abnormal swim bladders. Rotating drum tests confirmed that no radical changes in vestibular function had occurred, and no significant differences were found in subsequent light orientation tests. Tests of geotactic response in fish after 6 months or more of development suggested a tendency for the 32-hr flight fish to spend a significantly greater portion of their time in the upper portion of the test apparatus. Other treatment groups exhibited no statistically significant tendency, but in all groups tested there was evidence that the flight fish might be more sensitive to environmental factors.

Extensive light and electron microscopic examination of flight and control materials have revealed no significant differences in the embryological development of the central nervous system, peripheral vestibular apparatus, the eye or the cardiovascular system, of any of the animals examined thus far. The otoliths of flight juveniles were compared by scanning electron microscopy with otoliths from control animals maintained on Earth. The medial surfaces of the sagittae are characteristically grooved and sculptured; the lateral surfaces are smooth with concentric "growth rings" and "crystalline" projections in the groove in larger fish. None of the scanning electron microscopic examinations revealed recognizable differences in size, shape, or surface structure between flight and control juveniles. Histological and ultrastructural analyses of all age categories revealed that the peripheral vestibular system developed fully with complete expression

of the maculae of the sacculus and utriculus. The statoliths of the sacculus and utriculus followed normal sequence, and the lagenal statolith initiated development between posthatching days 4 and 6 in all groups. Except for a decrease in the extent and content of the vitreous cavity, ocular development was judged to be normal. Ultrastructural analyses of the developing statoliths and sensory maculae have revealed no differences between matched groups of animals. Both sustentacular and hair cells followed normal developmental parameters. Central projections and vestibular ganglia contained normal cellular complement and projection configuration. Cartilage development and calcification was identical in flight and control categories. Preliminary analysis of the specimens treated with tritiated thymidine at selected postsplashdown periods has also failed to reveal any significant alterations in the patterns of cellular proliferation within the vestibular, ocular, or central nervous systems of the oldest stages flown. Cells of the ependyma and cortical neuroepithelium, the planum semilunatum of the maculae, and the ora serrata of retina were heavily and consistently labeled in all groups.

EXPERIMENT DESCRIPTION

The main experiment package of the Cosmos K-104 experiment was based upon the design of the ASTP experiment and modified only to the extent necessary to conform to the new requirements and constraints of the Soviet spacecraft, launch/recovery procedures, and flight hardware. Each experimental unit consisted of a polyethylene bag containing 50 embryos of a given age and 23 ml sterile filtered 21‰ Instant Ocean. An experimental treatment contained 2 units each of five different embryo age groups as follows:

Unit	Calculated Launch Age	Average State of Development
1	32 hr	Mid-to-late gastrula
2	42 hr	Tubular profile of membranous labyrinth apparent. No evidence of statolith precursors
3	66 hr	Appearance of statolith precursors
4	88 hr	Initiation of ventral elongation of medial sensory patch. Initial elaboration of semi-circular canals
5	128 hr	Statoliths formed but without definitive margins

Age was based upon development time at 295 K (22 C). Each treatment group thus contained 100 embryos of each of the 5 different nominal development stages for a total of 500 eggs.

Experimental treatments were as follows:

K-1: Flight treatment, null-gravity (stationary mounted). Five hundred eggs as indicated above were fertilized, allowed to develop and then packaged, chilled, transported and flown as indicated in Table 1.

K-2: Flight treatment, one-gravity onboard control (centrifuge mounted). Eggs were treated identically with K-1.

K-3: Ground Control 1. Eggs were prepared and transported with K-1 and 2. At the time indicated in Table 1 the experiment package was mounted on a 5 rpm tube rotator and subjected to slow rotation (tumbling) in order to simulate the uniform dispersion of eggs in null-gravity.

K-4: Ground Control II. Eggs were prepared and transported with K-3. The flight package was allowed to remain in a stationary position. Ten eggs

Table 1

Environmental Conditions Encountered by Cosmos Experimental Embryos

Treatment Number	22°C Prechill Incubation Time for Embryos 128 hr	88 hr	66 hr	44 hr	32 hr	Chill Cycle	Temperature/Treatment Profile Transport Cycle 10°C	Warm-up	Flight	Recovery
K-1	108	68	46	24	12	1.75 hrs. (18°C-10°C)	65 hrs (drop to 8.6° for 4-5 hrs)	8 hrs 21°C	19½ day 21° 0-G	52 hrs 10°C
K-2	"	"	"	"	"	"	"	"	" Centrifuge	"
K-3	"	"	"	"	"	"	81 hrs (Temperature stable)	4 hrs 20°C	19¼ days 21°C Rotated Moscow	14 hrs 21°C
K-4	"	"	"	"	"	"	"	"	" Stationary Moscow	"
K-5	"	"	"	"	"	"	90 hrs	3 hrs 22°C	20 days 22°C USA Rotated	24 hrs 10°C
K-6	Continuous development at 22°C until hatching									

were removed from each development group and fixed at time of launch to provide a reference point for gauging the developmental stage at time of null-gravity exposure.

K-5: Ground Control U.S. Eggs were prepared identically with K-1 through K-4; transport was simulated and the flight package was mounted as for K-3.

K-6: Dish Control. Eggs were fertilized as for K-1 through K-5, but were allowed to continue development until hatching under standard environmental conditions in petri dishes.

EXPERIMENT HARDWARE

The experiment hardware consisted of four major items: the egg bags, the experiment container, the low temperature transporter and the outer flight container supplied by the Soviet side. The egg bag configuration was based upon that of the ASTP experiment. The same 2.6 mil clean room polyethylene was employed in fabrication but individual packets were used instead of the string of connected packets of the ASTP flight package. Dimensions of the flattened bags were 7.0 cm wide by 8.4 cm long inside the 2.5 mm heat seals. A double width heat seal was employed for final closure of the bag.

The experiment container was a two-chambered, machined aluminum, cuboidal case of 8.9 x 9.5 x 9.8 cm outside dimensions. Five egg bags were packed into each chamber sandwiched between thin perforated sheets of Pyrel(TM) foam. The case was closed by a perforated plate held in place by four Allen head screws.

The low temperature transporter consisted of an insulated aluminum case, approximate dimensions 35 x 40 x 45 cm with a central aluminum compartment for containment of the samples. The compartment was wound with heater wire. Current to the heater was furnished by dry cell batteries through a thermostatic control circuit. Between the sample chamber and the outside case, separated from both by layers of foam insulation, were flat containers of ice. The chilling capacity was supplied by the ice, restricted by the layer of insulation. This chilling effect was bucked by the heater circuit on the sample chamber. In operation this system allowed maintenance of 10 C $\pm$ 0.2 for a 72-hour period.

The Soviet supplied flight container was a sheet metal box fastened to a machined baseplate by means of screws. Screw holes in the baseplate mated with attachment points in the Soviet spacecraft. In operation the K-104 experiment container shared the flight container with another U.S. experiment container - the carrot cell experiment K-102. Both were held in place and protected by a preformed Pyrel foam filler.

DATA COLLECTION

The primary data yielded by the experiment was in the form of fixed material for light and electron microscopic analysis. Fixation was in the cold (ice bath) in Kalt-Tandler (Keefe, 1973) fixative. Samples were fixed at recovery and at intervals up to 30 days following recovery. The primary point of interest in all examinations was the vestibular and other sensory regions.

Postflight observation and testing were carried out to detect possible differences in orientation behavior attributable to the null-gravity exposure during development. The tests employed, light orientation, rotating striped drum and geotaxis test, have been described elsewhere (Scheld et al., 1975; Scheld et al., 1976) as they were employed for ASTP postflight testing. These same tests were used without significant modification.

EXPERIMENT EXECUTION

The basic procedure for preparation of fertilized eggs followed that of the ASTP experiment (Scheld et al., 1975). Specific timing for this experiment is indicated in Table 1. Eggs fertilized at the appropriate times prior to launch were allowed to develop to known stages of development and then sealed in the plastic packets and chilled to 10^{o}C to retard further development during transport to the launch site. For planning purposes it was assumed that elapsed time of chilled transport would be 72 hours and time from rewarming and loading of flight package to launch would be 8 hours. Progress of development at 10^{o}C for 72 hours was calculated to be equivalent to 12 hours at standard temperature of 22^{o}C. Fertilization of eggs was thus scheduled to produce embryos that at the time of chilling would be 20 hours younger than the expected launch ages.

Slow chilling of the eggs was effected by placing the sealed plastic bags in plastic beakers containing 1000 ml of water and allowing these to chill to 10^{o}C in a 6^{o}C incubator. The chilled bags were then quickly transferred to the equilibrated 10^{o} transporter and packed for shipment.

Transport and launch of the flight package proceeded without incident. In Moscow at approximately 36 hours before launch, control eggs were removed to a separate transporter and held in Moscow for treatment, while flight eggs were taken to the launch site. As indicated in Table 1, eggs were removed from the transporters a few hours prior to launch, loaded into the experiment hardware and then placed onboard the spacecraft.

At recovery flight-treated packets were removed from the flight containers, marked and then placed in the 10°C transporter for shipment to the Moscow laboratory. Ground controls were not subjected to this chilling treatment because access to these materials was delayed by airline scheduling, until nearly 1-1/2 days after recovery and chilling of the flight materials had occurred. This deviation was considered to be justified in light of baseline laboratory tests which have indicated that chilling has no harmful effect and because a chilling treatment of control eggs would have caused a considerable delay and complication of the recovery procedure.

An adjunct experiment carried out by Soviet investigators considered the effects of clinostatting upon development. Eggs were fertilized and prepared by the standard procedures to be chilled and shipped 10 days prior to launch. Development, at the time of chilling for shipment, had proceeded for 12 hours, 36 hours and 66 hours, respectively, in three separate sets of 300 eggs each. Upon arrival in the Soviet Union, eggs from the three age groups were randomly divided into treatment and control groups for clinostat exposure. Periodically during the course of development, samples were removed and fixed for microscopic examination. At approximately 36 hours prior to launch, subgroups of the treatments were chilled, transported, and launched with the eggs of the main experiment. Results of these experiments are summarized in Krasnov (1976).

RESULTS AND DISCUSSION

Recovery Activities

The experiment was carried out according to the timing indicated in Table 1 and in the "Experiment Execution" section of this report. Upon opening of the experiment packages severe retardation and high anomaly rates were apparent in all treatments except the Houston Standard Dish Control. The originally planned sampling and testing schedules were modified to allow for the slower hatching rates, and reduced numbers of samples. All eggs were examined under a binocular microscope and counts of normal (Table 2), abnormal (with types of abnormality indicated) and dead were recorded. Following microscopic examination, all eggs were shaken for 20 minutes on a rotary shaker and then examined for hatching 20 minutes or longer after the end of the shaker period. Except for the time in transit from the USSR, this routine was followed until all normal eggs had hatched.

Significant numbers of hatchlings did not emerge until the third and fourth days postflight. All morphologically normal hatchlings exhibited a typical fright-diving response and there were no apparent differences among the treatments with respect to diving response. No changes in orientation were noted in response to unidirectional light through the sides or bottoms of the containers. Beginning on the third day post-recovery, motion picture recordings were made of normal and disturbed swimming activity, geotactic response and orientation in a rotating striped drum.

Table 2

Normal Hatchlings Record from the Kosmos Mission*

Treatment	Age 32 hrs.	44 hrs.	66 hrs.	88 hrs.	128 hrs
K-1 (flight stationary)	6	4	1	14	38
K-2 (flight centrifuge)	4	10	6	11	27
K-3 (ground rotated)	1	1	0	0	1
K-4 (ground stationary)	0	2	0	0	25
K-5 (USA rotated)	0	0	0	0	0
K-6 (USA dish)	99	97	100	97	98

*Not corrected for hatchlings from any eggs given to Soviet investigators. All treatments contained 100 eggs except K-4 which contained 90.

Samples of hatchlings and eggs were fixed for microscopic examination on the fourth, seventh, and thirtieth days postflight. Major sampling was from the population of anomalous hatchlings. Only representative samples were taken from the apparently normal population. All samples were post-fixed with osmium and embedded in Epon plastic for sectioning.

Behavioral Testing

It is now a virtual certainty that development in weightlessness from the earliest exposure achievable under the current experimental constraints, or the additional tenure in space for 8-14 days past time of theoretical full embryonic development has no radical effect upon vestibular function. Further, it has become obvious from all observations thus far that alterations in behavior, if they exist, will be distinguishable only by relatively high-resolution quantitative techniques.

Preliminary investigations (Scheld et al., 1976) of geotactic response in maturing (6-8 months old) ASTP hatchlings have shown a statistically significant difference in tendency of 32-hr hatchlings, only, to swim predominantly in the upper half of the test apparatus. There was also some indication that flight fish in general were more sensitive to their environment. The significance of such subtle changes is difficult to assess without further data; the preliminary results were not confirmed because of loss of the flight animals in a laboratory mishap.

Histological Examination

Since all of the animals flown on this flight were impacted by a non-spaceflight factor that caused a 90-95% anomaly production in both flight and ground control groups (except open-dish control group), microscopic studies have been restricted to the 5-10% of the specimens adjudged to be grossly normal. Additional control specimens were obtained from earlier studies flown on the ASTP mission. In the present material lesions of various regions of the CNS (predominantly diencephalic) indicate a less than normal overall process of development. These lesions have been observed in all groups except the open dish controls and were never encountered in specimens derived from earlier control groups.

The peripheral vestibular apparatus develops in both normal time and proper sequence. Lateral, anterior and posterior semicircular canals originate from the closed otocysts in usual sequence (figs. 3, 4, 6, 9 and 11). The utricular and saccular maculae originate from a common primordium and follow tubular rotation to become fully differentiated. Both utricular (figs. 6, 9 and 10) and saccular (figs. 6 and 11) maculae frequently demonstrate "darkcells" (fig. 9). No differences were observed in the distribution or frequency of such cells between age-matched flighted and ground control specimens.

The sensory maculae develop hair cells (figs. 10 and 13) capped with a single kinocilium surrounded with 21 to 24 microvilli in normal sequence. The pairing basal body in the apex of each hair cell (see B in fig. 13) gives rise to a system of rootlets (see r in fig. 13) that extend into the basal region of each cell. The apical terminal web region of each hair cell and the basal synaptic regions differentiated in normal time sequence.

The otolithic membrane (OM) appears to be derived by a dual system of cellular secretion in this species: (1) a process of apocrine secretion in which apical portions of sustentacular cells are released into the otolithic membrane proper (see CV and V in fig. 13); and, (2) a separate discharge of secretory materials derived from the extensive rough endoplasmic reticulum (see R in fig. 13) and involving a single large Golgi complex. These observations suggest that there are either two populations of sustentacular cells or two states of the same cell type responsible for the initial elaboration of the otolithic membrane.

Both the utricular (Lapillus) and the saccular (Sagitta) statoliths condensed in concentric layers around an apparent initiation site which remains visible as a metachromatic dense granule(s) (arrows in figs. 9, 10 and 11). Appositional growth continued both in flight and upon recovery prior to sacrifice with no apparent alterations in the rates of accretion of statolithic substance. Concretion rings displaying typical metachromasia (arrow in fig. 11) were formed in similar positions in all specimens.

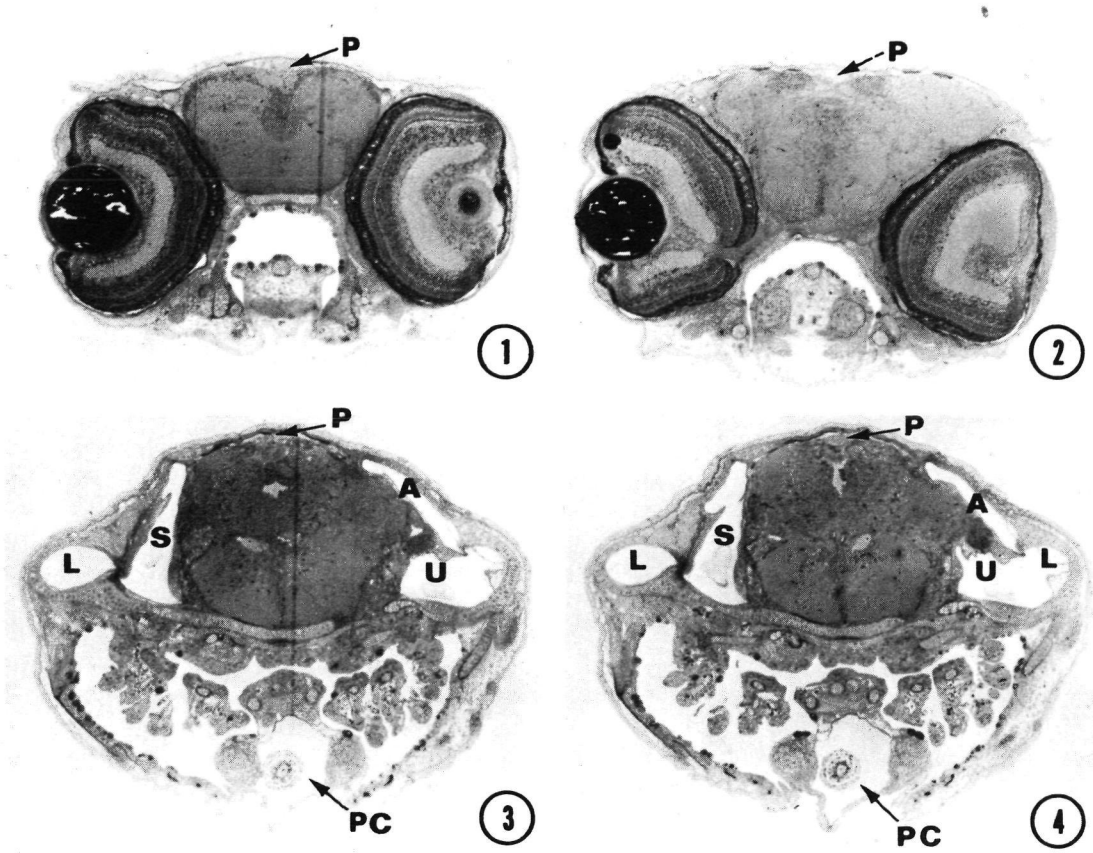
Further evidence of the normality of central nervous system development under spaceflight conditions is observed in the patterns of differentiation of both pineal and pituitary glands. The pineal consists of an elongated tubular profile with an enalrged terminal saccule (see P in figs. 1 thru 6 and PIN in fig 7). The pineal appears to be a secretory-type structure composed of columnar cells encompassing the central lumen and gives no evidence of any light sensitive structures in these young animals. The pituitary gland follows normal differentiation patterns with respect to cessation of labelling with ^{3}H-thymidine and termination of ontogenetic cell death. Regions of continuing

cellular proliferation were observed in the midline ependyma as well as in marginal areas of hypo- and epithalamic regions (see zones labelled with P on figs. 7 and 8). Continuing ontogenetic cell death was a further indicator of the progressive differentiation in these regions (see arrows in figs. 7 and 8).

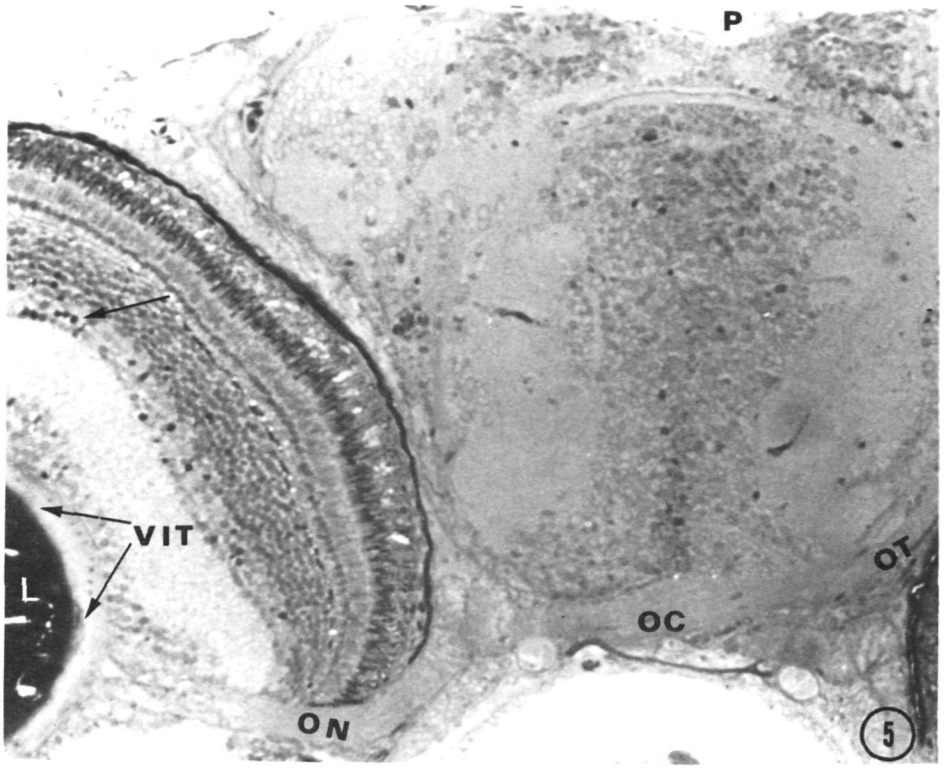
The neuropil of the central nervous system demonstrated normal vestibular projection profiles and central integrative structures. Such projection fibers could be followed due to their inherrent staining densities in the preparations of serial sections utilized (see asterisks in fig 11). One of the more readily observable and quantitated CNS structures are the Mauthner cell bodies with their prominent dendrites and myelinated terminal axons (see M and arrows in figs. 6 and 12). In all specimens examined the Mauthner cells were similar (within sample limitations) in size, position and dendrite distribution. Based upon light microscopic surveys of surface densities, no differences were observed in the patterns of synaptic terminations upon the dendritic surface.

The eye develops normally with all retinal layers demonstrating continued proliferative activity only at the ora serrata. Ontogenetic cell death continues to outline the developing plexiform layers (see arrow in fig. 5). The optic nerve (ON), optic chiasm (OC) and optic tract (OT) developed in normal time and density in all specimens.

An abnormality of the vitreous humor space, previously observed in similar studies flown on the ASTP mission, is demonstrated in figure 5. The near total absence of the vitreous space was considerably more pronounced in both groups of spaceflown fish. It is our belief that this deficiency of vitreous space reflects an underlying fluid/electrolyte problem which is also involved in the production of the pericardial edema obvious in the spaceflown fish (see PC in figs. 3 and 4). Although edematous alterations in the vascular channels supplying the vestibular apparatus have been observed in all spaceflown specimens, no alterations to endolymphatic fluids, the responsible secretory epithelia or their basement membranes (see asterisk on figs. 9 and 10) have been observed.



Figures 1-4:
Selected serial section derived from K-1 - 32hour specimen at 22 days postfertilization. A, anterior semicircular canal; L, lateral semicircular canal; P, pineal gland; PC, pericardial sac; S, sacculus; U, utriculus. All figures 100x magnification.



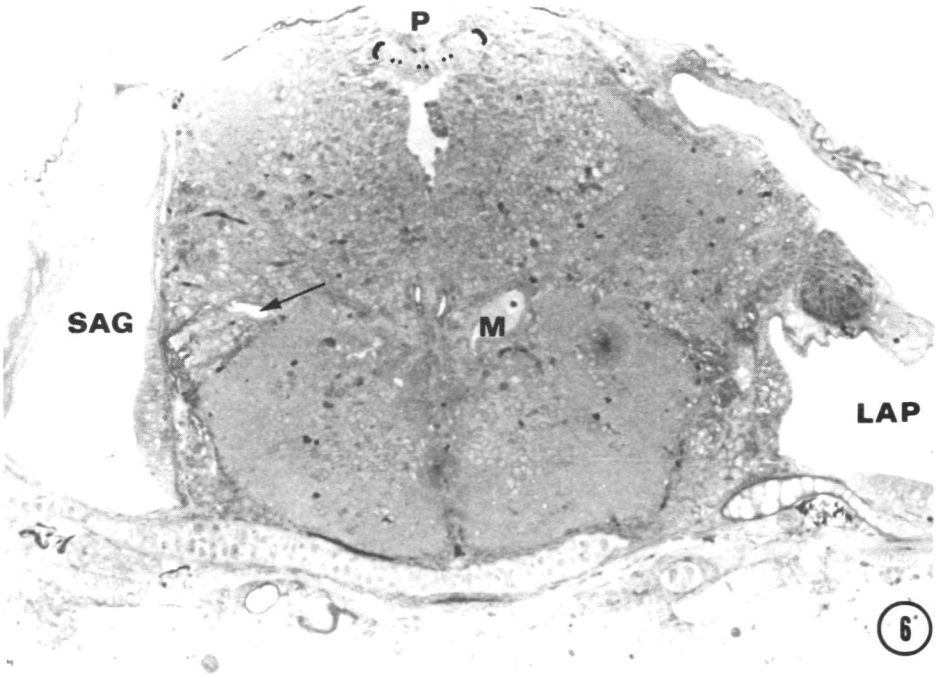
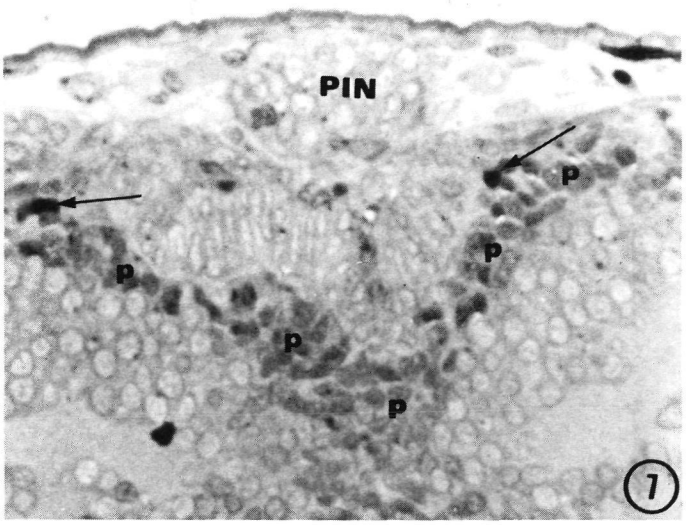


Figure 5: Detail through optic chiasm derived from Figure 2. ARROW, ontogenetic cell death on plexiform border; L, lens; OC, optic chiasm; ON, optic nerve; OT, optic tract; P, pineal gland; VIT, vitreous humor space. Magnification - 500x.

FIGURE 6: Detail of figure 4. LAP, lapillus (utricular statolith); M, Mauthner cell body (ARROW, Mauthner cell dendrite); P, pineal gland; (.. ....) epithalamic/parapineal primordium ; SAG, sagitta (Saccular statolith). Magnification - 300x.



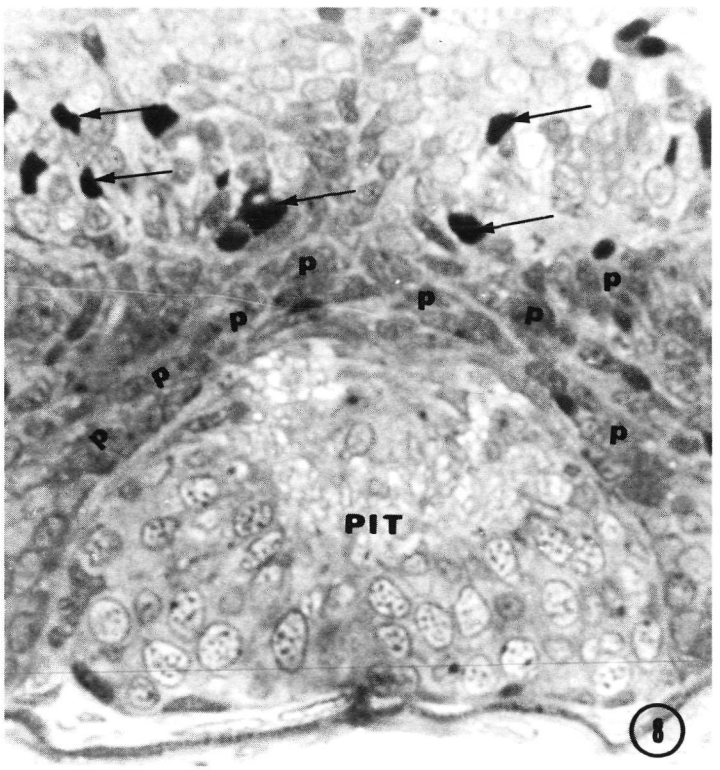
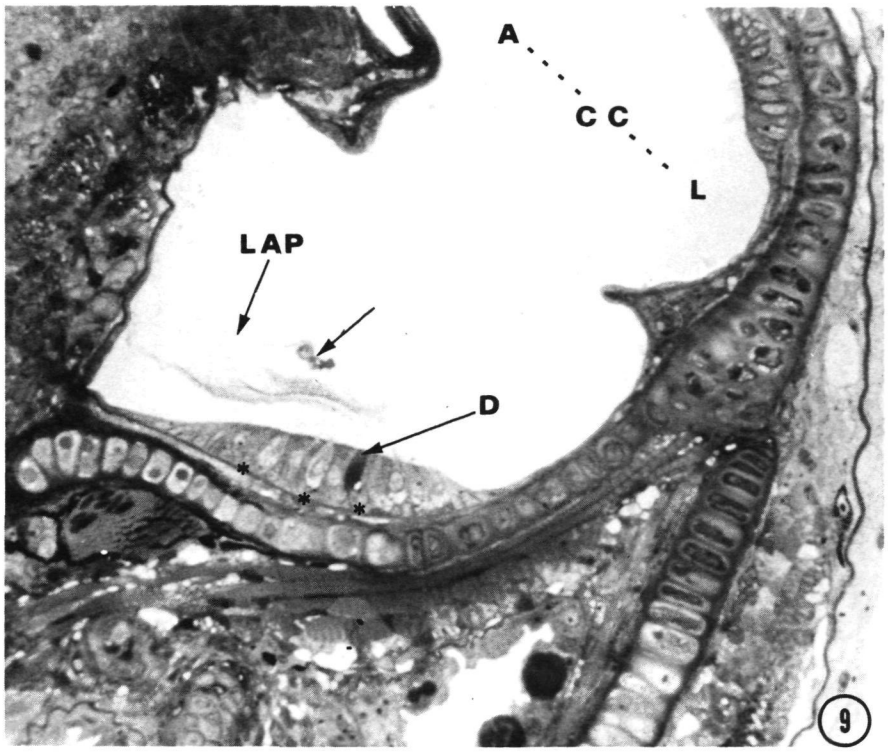


Figure 7: Detail through portion of tubular pineal gland of 32-hour flight animal. P, proliferative cell population in epithalamic region; PIN, tubular pineal gland; ARROWS, ontogenetic cell death. Magnification - 500x.

Figure 8: Detail of hypothalamic-hypophyseal region from a 32-hour flight animal. P, proliferative population in hypothalamic region; PIT, pituitary gland; ARROWS, ontogenetic cell death in differentiating zone. Magnification - 500x.



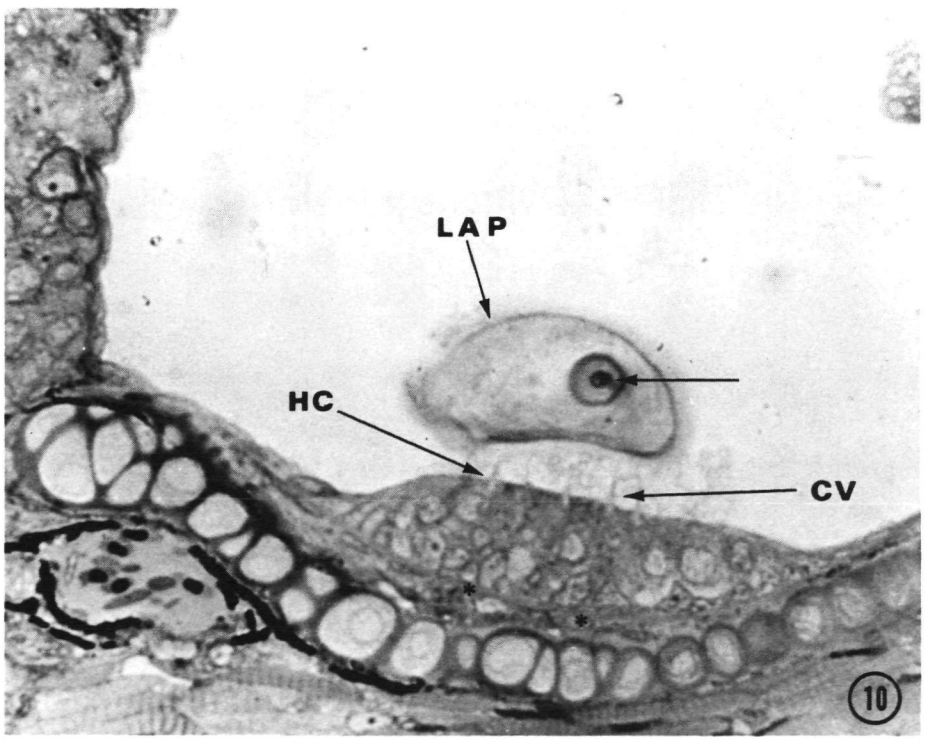
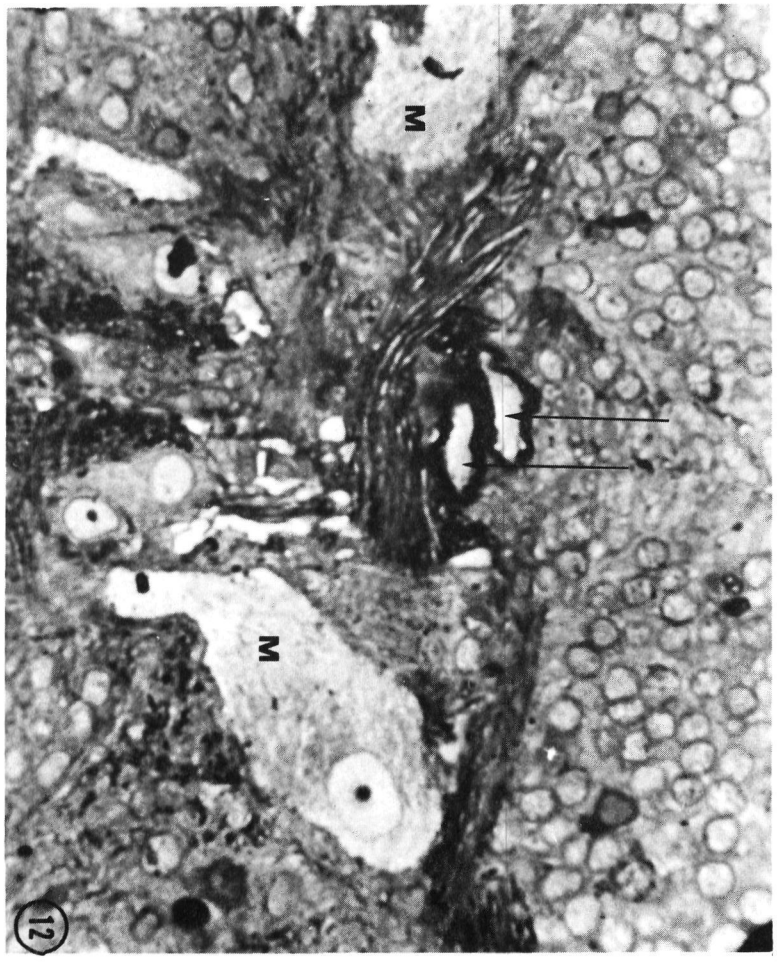


Figure 9: Detail of section through utricle of 32-hour flight animal. A---CC---L, anterior and lateral semicircular canals originating from common crus; ARROW, metachromatic dense granules in statolith; D, dark cell in macula; LAP, lapillus (utricular statolith); ASTERISKS, basement membrane of sensory macula. Magnification - 500x.

Figure 10: Detail of section through utriculus of 44-hour flight animal(one gravity centrifuge specimen). ARROW, metachromatic dense granule of statolith; ASTERISKS, basement membrane of sensory macula; CV, cytoplasmic vacuole forming otolithic membrane; HC, hair cell of sensory macula; LAP, lapillus (utriculae statolith). Magnification - 1000x.



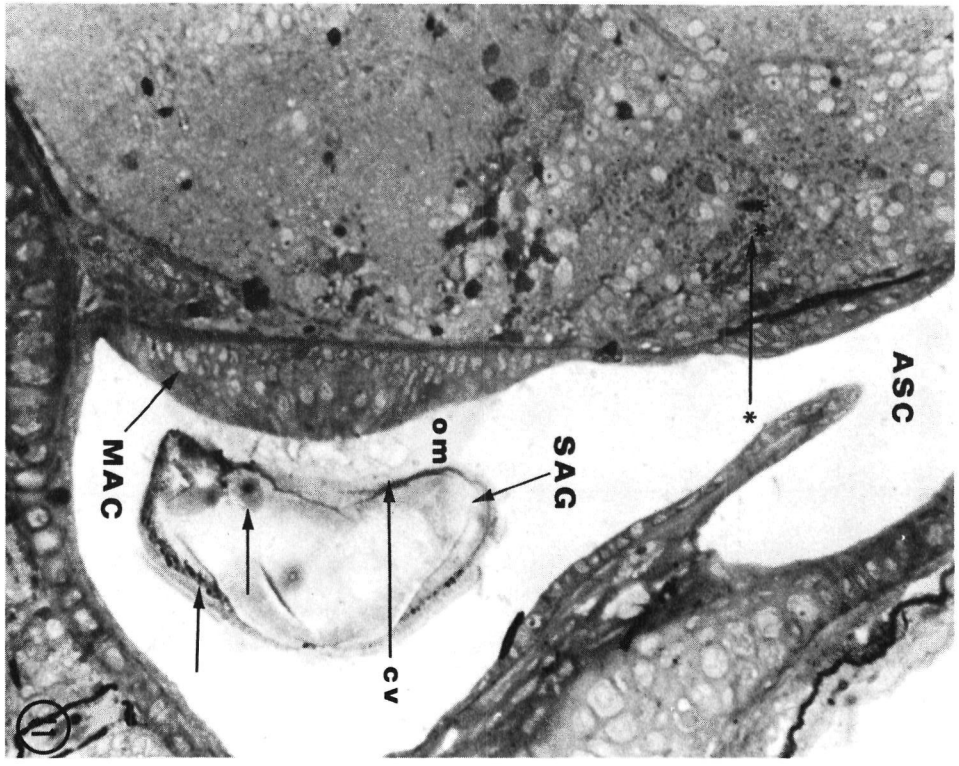


Figure 11: Detail of section through sacculus of 44-hour flight animal (one gravity centrifuge specimen). ARROWS, metachromatic dense granule and accretion line of statolith; ASC, anterior semicircular canal; ASTERISK, cross section of vestibular nerve root; CV,cytoplasmic vacuole forming otolithic membrane; MAC, sensory macula of sacculus;OM, otolithic membrane; SAG, sagitta (saccular statolith). Magnification - 1000 x.

Figure 12: Detail through central neuropil surrounding Mauthner cells from a 44-hour flight animal (one gravity centrifuge specimen). M, Mauthner cell body; ARROWS, Mauthner cell axons. Magnification-1100x.

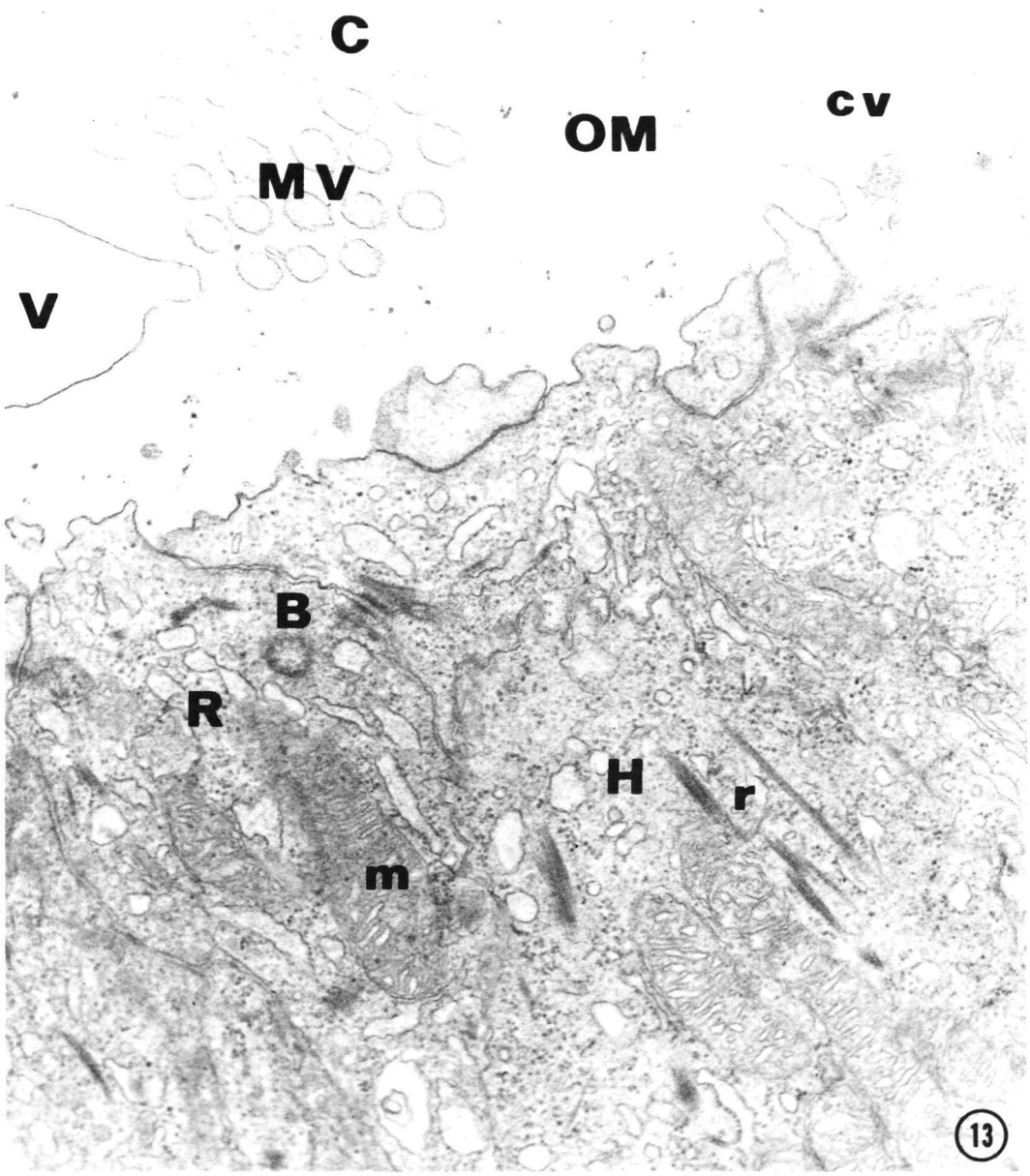


Figure 13: Electron micrograph of apical portion of sensory macula from the Utricle of a 128-hour flight animal. B, basal body; C, sensory kinocilium; CV, cytoplasmic vacuole forming otolithic membrane; H, hair cell of sensory macula; M, mitochondrion of sustentacular cell; MV, microvilli of hair cell surrounding kinocilium; OM, otolithic membrane between apical surface of sensory macula and statolith (not shown); R, distended cisternae of rough endoplasmic reticulum in sustentacular cell; r, ciliary rootlets of hair cell; V, apparently empty cytoplasmic vacuole. Magnification - 20,000x.

Cause of Anomalous Development

Postflight testing of procedures and materials from the experiment indicates that the probable cause of the high incidence of anomalous development lies in the tape used to label the plastic bags. Tests have shown high anomalies even in unlabelled bags immersed in water containing labelled bags. Analysis of organic volatiles from the tape by gas chromatography/mass spectrometry has resolved only one major component, carbon disulfide, that has known toxic properties in the concentration range potentially available in the experimental packages. All other detectable volatile components were present in much lower concentration and none have known toxic properties. Tests with carbon disulfide have demonstrated the capability for causing death or delay and anomalous development in Fundulus embryos.

CONCLUDING REMARKS

The experiment series to which the present experiment belongs was begun to explore the possible deleterious effects of exposure to weightlessness upon development and growth of biological systems. The results obtained thus far from these experiments are very encouraging in that we can say with considerable confidence that development of Fundulus beyond the gastrula stage is not affected in any major way by weightlessness. At this point it seems somewhat doubtful that we will discover very many minor effects, and it is tempting to speculate that weightlessness may be largely beneficial.

Most discussions of effects of weightlessness upon development appear to assume the weightless state to be a stressful condition. Practical experience with Fundulus development would suggest the opposite. There was indication of generally better health in flight animals of all three experiments of this series. With the possible exceptions of those aspects of development in which gravity is required as a cue for establishment of polarity or as a reference stimulus for sensory development, it is probable that the weightless state provides generally superior conditions for embryo development. This may not be true for situations in which maternal-fetal interaction is part of the process, but even there, the source of stress would be through the parents interaction with the environment while stress on the embryo and stress on the parent resulting directly from pregnancy should be reduced. Within the limits already noted, (i.e., establishment of polarity) there would seem to be no reason why earlier development of Fundulus from fertilization through gastrula should not also be enhanced. It is recommended that future experiments with the Fundulus be designed with this aspect as a prime consideration.

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